Biochemical Effect of Cranberry Extract on Experimental Toxicity with Iron

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A B S T R A C T

The present study was aimed to evaluate the protective effective role of cranberry extract against iron-overload induced hepatic toxicity in rats. By administration of iron sulfate (30 mg/kg body weight) orally for 21 days, led to significantly increases in the levels of hemoglobin, glucose, lipid profile, plasma and hepatic lipid peroxidation, iron and transferrin. Also, the oral administration of iron sulfate showed a significant decrease in the levels of plasma and liver enzymatic and non-enzymatic antioxidants. Administration of cranberry extract at different doses (75 and 150 mg/kg body weight) significantly restore the levels of plasma and hepatic markers, lipid profile, lipid peroxidation as well as increased the levels of plasma and hepatic antioxidants with normal plasma iron and transferrin concentration in blood. Cranberry extract at a dose of 150 mg/kg body weight exhibits significant protection on hepatic antioxidants more pronounced than both cranberry 75 mg/kg.b.w. and vitamin C. On the other hand, the results clearly suggest that the cranberry extract may be effective in enhances the protection of liver toxicity by its free radical scavenging effect and antioxidant activity.

Key Words: Cranberry extract, Iron overload, liver antioxidant, hepatic oxidative stress biomarkers.

1. INTRODUCTION

Iron is a nutrient related to health and immunity (Heidarpour B., et al., 2008). It is the most common element on earth, is chemically unstable and easily oxidized into an insoluble ferric form, ferric iron is unavailable in most biological systems (Mohri, M. et al., 2006). As a transition metal, iron has five oxidation states (Fe^{2+}–Fe^{6+}) in addition to the ground state, and the most common states are Fe^{2+} and Fe^{3+} (Henle, E. S. and Linn, S. 1997). The unpaired electrons from iron make one-electron redox reactions possible. Fenton discovered that ferrous iron and hydrogen peroxide catalyze the oxidation of tartaric acid (Koppenol, W. 1993, and Fenton, H. 1894). The study of Haber and Weiss subsequently showed that the hydroxyl free radical was produced by ferrous iron and hydrogen peroxide in a chain reaction, as shown below (Walling, C. 1993, Haber, F. and Weiss, J. 1934). The OH radical can non-specifically oxidize lipid molecules in the cell membrane and lipoprotein lipids (e.g., unsaturated fatty acids in phospholipids and cholesterol esters) to form LOOH in the Fenton reaction (Fenton, H. 1894). Further, Fe^{2+} can catalyze the single-electron reduction of LOOH, the non-radical lipid hydroperoxide, to produce the LO (OLOO) free radical via one-electron oxidation (Girotti, A. 1998, Cheng, Z. and Li, Y. 2007). Lipid peroxidation is initiated at this step. Some studies showed that ferric iron seems necessary to initiate lipid peroxidation (Minotti, G. and Aust, S. D. 1987, Minotti, G. and Aust S. D. 1987) or some Fe^{4+} or Fe^{5+} complexes could be formed to initiate lipid peroxidation (Goldstein, S. et al., 1993). Thus, free

radicals result in the consumption of antioxidant defenses which may lead to disruption of cellular functions and oxidative damage to membranes and enhance susceptibility to lipid peroxidation. Increased generation of reactive oxygen species (ROS) and lipid peroxidation has been found to be involved in the pathogenesis of many diseases of known and unknown etiology and in the toxic actions of many compounds (Andallu, B. and Varadacharyulu, N. 2003). Antioxidants play an important role to protect the human body cells against damage caused by reactive oxygen species (Baynes, J.W. 1991). The endogenous antioxidant enzymes (e.g SOD, CAT, GSH and GPx) are responsible for the detoxification of deleterious oxygen radicals (Jacob, R. 1995). Flavonoids are phenolic compounds abundantly distributed in plants. It has been reported that most of them had effective antioxidants (Rice-Evans, CA. et al., 1996). Many plant extracts and plant products have a significant antioxidant activity (Anjali, P. and Manoj, K. M. 1995), which may be an important property of medicinal plants associated with the treatment of several ill-fated diseases as diabetes. Thus, herbal plants are useful means prevent and/or ameliorate certain disorders, such as diabetes, atherosclerosis and other complications (Scartezzini, P. and Speroni, E. 2002). One of these plants, Cranberry berry ranks high among fruit in both antioxidant quality and quantity (Vinson, JA. et al., 2001) because of its substantial flavonoid content and a wealth of phenolic acids. Cranberry extracts rich in these compounds reportedly inhibit oxidative processes including oxidation of low-density lipoproteins (Yan, X. et al., 2002, Porter, ML. et al., 2001), oxidative damage to at neurons during simulated ischemia (Neto, CC. et al., 2005), and oxidative and inflammatory damage to the vascular endothelium (Youdim, KA.et al., 2002). The antioxidant properties of the phenolic compounds in cranberry fruit may contribute to the observed anti-cancer activity. Plant-derived fractions are rich sources of phenolic compounds (Ebrahimzadeh, MA. et al., 2009). Phenolics are known to have potential role to prevent tumor and have been used in aromatherapy for obese middle-aged women. Flavonoids extracted from plants may have antioxidant activity that could mitigate tumor-related complications, including atherosclerosis and some cancers (Ebrahimzadeh, MA. et al., 2009, Han, SH. et al., 2003). Not surprisingly, Cranberry extract contain high levels of unsaturated fatty acids and poly-phenols (Yan, X. et al., 2002, Ebrahimzadeh, MA. et al., 2009), which are excellent scavengers of reactive and represent a promising iron chelating effects. In vivo tests have been conducted with foods to determine, its hepatoprotective (Scartezzini, P. and Speroni, E. 2002), hypolipidemic, hypoglycemic and antioxidant activity (Vinson, JA. et al., 2001). As a continuation of interested research program in pharmaceutical importance of natural products (Abdel-Maksoud, HA. et al., 2015, Hussein, MA. And Abdelgwad, SM. 2010) especially cranberry extract (Abdel-Maksoud, HA. et al., 2015, Abdel-Maksoud, HA. 2015), we report here in, a facile route to evaluate the chelating, antioxidant and hepatoprotective effects of cranberry extract against oxidative stress induced by iron sulfate overload in rats.

2. MATERIALS AND METHODS

2.1. Dose of Cranberry:

Cranberry extract was purchased from Virgin Extracts (TM), Chinese. **Cranberry** was given to adult rats with 1/150 LD50 (75 mg/kg.b.w.) and 1/75 LD50 (150 mg/kg.b.w.) daily for 3weeks (Abdel-Maksoud, HA. et al., 2015, Abdel-Maksoud, HA. 2015) by oral gastric gavage tube. Ferrous sulfate 99% was purchased from Sigma Aldrich, USA. CO.

2.2. Experimental animals:

This experiment was occured in accordance with guidelines established by the Animal Care and use Faculty of Pharmacy, October 6th University, Cairo, Egypt. 50 adult rats weighting around 180 ± 10 gms were purchased from Faculty of Veterinary Medicine, Cairo University. They were individually housed in cages in an air-conditioned room with a temperature of 22 $\pm$ 2ºC, a relative humidity of 60%, and an 8:00 to 20:00 light cycle. During the acclimatization period, each animal was raised on a regular diet *adlibitum*. The regular diet consists of wheat flour 22.5%, corn starch 27.2%, soybean powder 20%, essential fatty acids 0.6%, sucrose 10%, cellulose 2.6, corn oil 5%, vitamins 2% (A 0.6 mg/kg, D 1000 IU/ kg, E 35 mg/kg, niacin 20 mg/kg, pantothenic acid 8 mg/kg, riboflavin 0.8 mg/1000 kcal, thiamin 4 mg/kg, B6 50 µg/kg and B12 7 mg/kg of diet) and minerals 10% (calcium 5 g/kg, Phosphorus 4 g/kg, fluoride 1 mg/kg, iodine 0.15 mg/kg, chloride 5 mg/kg, iron 35 mg/kg, copper 5 mg/kg, magnesium 800 mg/kg, potassium 35 mg/kg, manganese 50 mg/kg and sulfur 3 mg/kg of diet) (NRC 1996).

2.3. Experimental design:

The animals were divided into 5 groups consisting of 10 Rats (180 ± 10 gm), two controls groups and three treatment groups: Group (1): Control negative (0.9% saline, 3 ml/kg.b.w., orally). Group (2): Positive control (ferrous sulfate 30 mg/kg.b.w. suspended in 1 ml 0.9% saline was given orally daily for 21days) (Leelavinothan, P. et al., 2015, Akira, Y. and Kimiko, H. 2004). Group (3): Ferrous sulfate 30 mg/kg.b.w. + Cranberry extract (75 mg/ kg.b.w.) daily for 21 days, orally daily dose (Abdel-Maksoud, HA. et al., 2015). Group (4): Ferrous sulfate 30 mg/kg.b.w. + Cranberry extract (150 mg/ kg.b.w.) daily for 21 days, orally daily dose (Abdel-Maksoud, HA. et al., 2015). Group (5): Ferrous sulfate 30 mg/kg.b.w. + Vitamin C (1 g/kg.b.w.) daily for 21 days, orally daily dose (Luo, Z. et al., 1995).

2.4. Blood and tissue samples:

At the end of the experiment, rats of each group were sacrificed by cervical decapitation. Blood samples were collected in dry, clean, and screw capped heparin tubes and divided in to two parts, one part for hemoglobin determination, Also, the second part was for plasma which separated by centrifugation at 2500 r.p.m for 15 minutes and kept in a deep freeze at (-20 °C) until used for subsequent biochemical analysis. The abdomen was opened and the liver specimen was quickly removed and cleaned by rinsing with ice-cold saline to remove any blood cells, clots, then blotted between 2 filter papers and quickly stored in a deep freezer at (-20 °C) for subsequent biochemical estimation of plasma and liver reduced glutathione GSH (Nichans, WH. And Samulelson, B. 1968), superoxide dismutase (SOD) (Chanarin, I. 1989), catalase (CAT) (Marklund, S. and Marklund, D. 1974) and TBARS (Sinha, AK. 1972) as well as blood hemoglobin (van Kampen, E. and Zijlstra, WG. 1961), plasma glucose (Trinder, P. 1969), iron (Ceritti, F. and Ceriotti, C. 1980), transferrin (Hellsing, K. 1973), alanine aminotransferase (ALT) (Reitman, S. and SA. Frankel, S. 1957), aspartate aminotransferase (AST) (Reitman, S. and Frankel, SA. 1957), alkaline phosphatase (ALP) (Kind, PRN. and King, EJ. 1954), triglyceride (Allain, CC. et al., 1974), total cholesterol (Fossati, P. and Prencipe, L. 1982), HDL- C (Burnstein, M. et al., 1970) and LDL-cholesterol (Falholt, K. et al., 1973). Plasma LDL-cholesterol level was calculated from Falholt and Falholt (Falholt, K. et al., 1973) formula (LDL-cholesterol = total cholesterol – triglycerides/5 – HDL-cholesterol).

2.5. Statistical analysis:

The obtained data were analyzed and graphically represented using the statistical package for social science (SPSS, 13.0 software, 2009), for obtaining mean and standard deviation of mean. The data were analyzed using one-way ANOVA to

determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparison among the groups for testing the inter-grouping homogeneity (Abo-Allam, R.M. 2003).

3. RESULTS

Oral administration of ferrous sulfate (30 mg/kg body weight) resulted in a significant increase in plasma glucose and blood hemoglobin compared to the normal control group ($p< 0.05$). Supplementation of cranberry extract at 75 and 150 mg/k.g.b.w. normalize plasma glucose and blood hemoglobin compared to the group that received ferrous sulfate (table 1). Oral administration of Ferrous sulfate (30mg/kg body weight) resulted in a high significant increase in plasma iron, transferrin and transferrin saturation (TS%)compared to the normal control group ($p< 0.01$). Supplementation of cranberry extract at 75 and 150mg/k.g.b.w. resulted in a significant decrease in plasma iron, transferrin and transferrin saturation (TS%) compared to the group that received ferrous sulfate ($p< 0.01$) (table 2). Oral administration of ferrous sulfate (30 mg/Kg.b.w.) resulted in a significant increase in plasma ALT, AST, and ALP compared to the normal control

group ($p< 0.01$). Supplementation of cranberry extract at 75 and 150 mg/k.g.b.w. resulted in a significant decrease in plasma ALT, AST and ALP compared to the group that received ferrous sulfate ($p< 0.01$) (table 3). Oral administration of ferrous sulfate (30 mg/Kg.b.w.) resulted in a significant increase in plasma total cholesterol (TC), triglycerides (TG) and LDL-C as well as a significant decrease in plasma HDL-C compared to the normal control group ($p< 0.01$). Supplementation of cranberry extract at 75 and 150 mg/k.g.b.w. resulted in a significant decrease in plasma total cholesterol (TC), triglycerides (TG) and LDL-C as well as a significant increase in plasma HDL-C compared to the group that received ferrous sulfate ($p< 0.01$) (table 4). Oral administration of ferrous sulfate (30 mg/kg.b.w) resulted in a significant decrease in blood and liver reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and catalase (CAT) as well as a significant increase in plasma and liver TBARs compared to the normal control group ($p< 0.01$). Supplementation of cranberry extract at 75 and 150 mg/k.g.b.w. resulted in a significant increase in blood and liver GSH, SOD and CAT as well as a significant decrease in plasma and liver TBARs compared to the group that received ferrous sulfate ($p< 0.01$) (tables 5 and 6).

Table 1: Plasma level of plasma glucose and blood hemoglobin in normal and experimental groups of rats.

No.	Groups	Glucose (mg/dL)	Hb % (g/dL)
(I)	Normal 0.9% saline	84.46± 5.11	12.70 ± 0.62
(II)	Control: Ferrous sulfate (30mg/Kg.b.w.)	137.18± 7.84[*a]	16.25± 0.50[*a]
(III)	Cranberry extract 75 mg/kg.b.w.	124.64± 8.25[*ab]	13.40± 0.43[*ab]
(IV)	Cranberry extract 150 mg/kg b.w.	115.37± 10.06[*abc]	12.86± 0.70[*abc]
(V)	Vitamin C 1 g/kg,b.w	110.66± 8.50[*abc]	12.75±0.68[*abc]

Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at *p< 0.01*. a: significant from normal control; b: significant from ferrous sulfate (30 mg/Kg.b.w.) supplement group; c: significant from cranberry extract (75 mg/kg.b.w.); d: significant from cranberry extract (150 mg/kg b.w.).

Table 2: Plasma level of plasma glucose, iron, transferrin and transferrin saturation (TS%)in normal and experimental groups of rats.

No.	Groups	Iron (µg/dL)	Transferrin (mg/dL)	Transferrin saturation (TS%)
(I)	Normal 0.9% saline	157.08± 9.80	183.74± 10.52	60.90 ± 4.38
(II)	Control: Ferrous sulfate (30mg/Kg.b.w.)	311.50± 8.35*a	246.19±18.73*a	90.13±5.08*a
(III)	Cranberry extract 75mg/kg.b.w.	208.22± 11.2*ab	205.20± 12.66*ab	72.29±3.40*ab
(IV)	Cranberry extract 150mg/kg b.w.	168.00± 6.18*abc	193.84± 8.47*bc	61.74±4.52*bc
(V)	Vitamin C 1g/kg,b.w	173.50±7.69*abc	188.33±9.58*abcd	65.63±3.95*b

Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at $p< 0.01$. a: significant from normal control; b: significant from ferrous sulfate (30 mg/Kg.b.w.) supplement group; c: significant from cranberry extract (75 mg/kg.b.w.); d: significant from cranberry extract (150 mg/kg b.w.). ST%= (Iron/Transferrin) * 71.24.

Table 3: Activity of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in plasma of normal and experimental groups of rats.

No.	Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
(I)	Normal 0.9% saline	18.76± 2.37	32.08± 2.00	68.27 ± 5.49
(II)	Control: Ferrous sulfate (30mg/Kg.b.w.)	42.38± 3.90*a	58.90± 5.82*a	105.40±8.73*a
(III)	Cranberry extract 75mg/kg.b.w.	30.21± 2.28*ab	44.73± 5.04*ab	83.29± 6.30*ab
(IV)	Cranberry extract 150mg/kg b.w.	23.86± 3.06*abc	36.08± 4.23*cb	71.25± 5.00*bc
(V)	Vitamin C 1g/kg,b.w	27.50± 3.77*ab	40.66± 4.39*b	79.64± 6.11*abd

Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at $p< 0.01$. a: significant from normal control; b: significant from ferrous sulfate (30mg/Kg.b.w.)supplement group; c: significant from cranberry extract (75 mg/kg.b.w.); d: significant from cranberry extract (150 mg/kg b.w.).

Table 4: Level of plasma total cholesterol (TC), triglycerides (TG), HDL-C and LDL-C of normal and experimental groups of rats.

No.	Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
(I)	Normal 0.9% saline	95.50 ± 6.48	77.20 ± 5.45	32.66 ± 3.28	47.78 ± 4.09
(II)	Control: Ferrous sulfate (30mg/Kg.b.w.)	164.66 ± 9.80^{*a}	142.33 ±13.26^{*a}	20.95 ± 2.07^{*a}	115.25 ± 10.79^{*a}
(III)	Cranberry extract 75mg/kg.b.w.	135.82 ± 11.37^{*ab}	119.70 ±11.15^{*ab}	28.36 ± 3.14^{*ab}	83.52 ± 6.42^{*ab}
(IV)	Cranberry extract 150mg/kg b.w.	107.15 ± 8.64^{*abc}	85.41 ± 6.58^{*bc}	36.24 ± 4.08^{*bc}	53.83 ± 3.09^{*bc}
(V)	Vitamin C 1g/kg,b.w	117.54 ±7.50^{*abcd}	98.05 ±6.44^{*abc}	31.18 ± 2.60^{*b}	66.75 ± 5.22^{*abcd}

Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at $p< 0.01$. a: significant from normal control; b: significant from ferrous sulfate (30mg/Kg.b.w.) supplement group; c: significant from cranberry extract (75 mg/kg.b.w.); d: significant from cranberry extract (150 mg/kg b.w.). LDL-C (mg/dl) = TC-HDL- [TG / 5].

Table 5: Level of blood reduced glutathione (GSH) and activities of superoxide dismutase (SOD), catalase (CAT) and Thiobarbaturic acid reactive substances (TBARs) in normal and experimental groups of rats.

No.	Groups	GSH (mg %)	SOD (U/mL)	CAT (U/mL)	TBARs (mmol/dL)
(I)	Normal 0.9% saline	23.50± 2.47	39.08± 3.00	25.97± 4.20	0.42± 0.05
(II)	Control: Ferrous sulfate (30mg/Kg.b.w.)	13.82± 2.09^{*a}	27.16±4.10^{*a}	9.18± 1.84^{*a}	0.67± 0.04^{*a}
(III)	Cranberry extract 75mg/kg.b.w.	19.00± 3.25^{*ab}	34.50± 4.28^{*a}	15.66± 3.05^{*ab}	0.55± 0.05^{*ab}
(IV)	Cranberry extract 150mg/kg b.w.	24.06± 3.17^{*bc}	41.29± 4.08^{*b}	23.98± 2.44^{*bc}	0.43± 0.06^{*bc}
(V)	Vitamin C 1g/kg.b.w	21.44± 2.76^{*b}	33.45± 2.68^{*abd}	20.86± 3.10^{*abc}	0.47± 0.04^{*ab}

Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at $p< 0.01$. a: significant from normal control; b: significant from ferrous sulfate (30mg/Kg.b.w.) supplement group; c: significant from cranberry extract (75 mg/kg.b.w.); d: significant from cranberry extract (150 mg/kg b.w.).

Table 6: Level of liver reduced glutathione (GSH) and activities of superoxide dismutase (SOD), catalase (CAT) and Thiobarbaturic acid reactive substance (TBARs) in normal and experimental groups of rats.

No.	Groups	GSH (μg/mgprotein)	SOD	CAT	TBARs (mmol/g tissue)
(I)	Normal 0.9% saline	5.2 ± 0.57	8.38 ± 1.22	74.16 ± 6.20	11.37 ± 1.27
(II)	Control: Ferrous sulfate (30mg/Kg.b.w.)	$2.35\pm 0.33^{*a}$	$4.70\pm0.84^{*a}$	$53.00\pm 4.09^{*a}$	$26.04\pm 3.75^{*a}$
(III)	Cranberry extract 75mg/kg.b.w.	$3.75\pm 0.41^{*ab}$	$6.32\pm 0.65^{*ab}$	$66.48\pm 4.28^{*ab}$	17.83 ± 2.09^{ab}
(IV)	Cranberry extract 150mg/kg b.w.	$4.94\pm 0.38^{*bc}$	$8.15\pm 0.43^{*bc}$	$72.06\pm 6.15^{*bc}$	$12.74\pm 0.86^{*bc}$
(V)	Vitamin C 1g/kg,b.w	$4.80\pm 0.50^{*bc}$	$7.05\pm 0.92^{*abcd}$	$68.11\pm 3.61^{*b}$	$15.27\pm 1.30^{*ab}$

Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at *p< 0.01*.SOD; one unit of enzyme activity was taken as the enzyme reaction, which gave 50% inhibition of pyrogallol autoxidation in one minute/mg protein; CAT; μmol of H_2O_2 consumed/min/mg protein; a: significant from normal control; b: significant from ferrous sulfate (30mg/Kg.b.w.) supplement group; c: significant from cranberry extract (75 mg/kg.b.w.); d: significant from cranberry extract (150 mg/kg b.w.).

4. DISCUSSION

Iron deficiencies or iron overload disturb the biochemical-physiological balance and are common having clinical relevance. To avoid toxic potential while still providing essential biological needs in anaemia, the levels of iron supplied must be rigorously controlled. The control of iron absorption is a key step in the process, because the metal absorbed is retained in liver and its excretion is limited (P.T. Lieu, 2001). However, iron accumulation after an overdose or a prolonged chronic therapeutic dosage suggests that a first order passive process could explain iron absorption in addition to physiologically non oxidative stress controlled. Acute iron overload induced significant deposition of iron in organs associated with exacerbated oxidative stress status and remarkable alterations of antioxidants. (Matsuura, 1983) demonstrated iron uptake by rat's liver and induction of hepatic iron loading and iron toxicity in the liver after a single injection of iron. Also, (Whittaker and Chanderbhan, 2001) reported that feeding

rats diets supplemented with carbonyl iron resulted in dose related increases in liver non heme iron and lipid peroxidation. Moreover, (El-Maraghy et al., 2009) demonstrated that Fe^{+3} overload induced a significant deposition of iron in rat liver associated with oxidative stress and a remarkable increase in hepatic nitric oxide level. Antioxidants are the first source of protection of the body against free radicals and other oxidants, being the compounds that the attack and the formation of radical species within cells. The group of antioxidants inside the organism is known as the total antioxidant state (TAS) (Teixeira, M. et al., 2013). The present study results, showed that oral administration of ferrous sulfate (30 mg/kg body weight) resulted in a significant increase in plasma glucose and blood hemoglobin compared to the normal control group. Supplementation of cranberry extract at 75 and 150 mg/k.g.b.w. normalize plasma glucose and blood hemoglobin levels compared to the group that received ferrous sulfate. These results were in agreement with previous studies by

(Baustad, B. and S. 1996, Wenzlf, O. and Erhardt, G. 1991) who concluded that iron administration as $FeSO_4$ provided an increase in RBCs parameters in rats. The data of the present study, showed that oral administration of Ferrous sulfate (30 mg/kg body weight) resulted in a significant increase in plasma iron, transferrin and transferrin saturation (TS%) compared to the normal control group. And the supplementation of cranberry extract at 75 and 150 mg/k.g.b.w. resulted in a significant decrease in plasma iron, transferrin and transferrin saturation (TS%) compared to the group that received ferrous sulfate.

These results were in agreement with (Sarker et al., 2013) who reported that mice injected with Fe^{+3} exhibited an increased in liver iron deposition. Administration of polyphenols significantly reduced serum and tissue iron concentration, these may be attributed to iron chelator effect of polyphenols. In addition, rats fed cranberry extract showed decreased level of liver ferritin, indicative of decreased iron burden (Jiao, Y. et al., 2006). Cranberry extract contained significant levels of vitamin C, total phenols, catechins and anthocyanins with associated antioxidant activity. Anthocyanins present in cranberry can also prevent the oxidation of ascorbic acid caused by metal ions through chelating the metal ions, and forming ascorbic (copigment)-metal anthocyanin complex (Sarma, A. D. and Sharma, R. 1999), and scavenge O_2 (Noda, Y. et al., 1998). Results of the present study, showed that a significant increase in plasma ALT, AST, and ALP compared to the normal control group. Supplementation of cranberry extract. resulted in a significant decrease in plasma ALT, AST and ALP compared to the group that received ferrous sulfate. In the present study, higher activities of plasma, ALT, AST, (an indicator of hepatocytes damage) have been found in response to iron overload-induced oxidative stress. Such increased activities might be attributed to the leakage of these enzymes from the injured liver cells into the blood stream because of the altered liver membrane permeability (Shohda, A. et al., 2009). Increase in serum ALP activities is the indicative of cellular damage due to loss functional integrity of cell membranes. The accumulation of iron in blood was effectively reduced by hesperidin, which revealed that cranberry's polyphenols chelate the iron. Moreover, the hydroxyl groups of polyphenols or its active metabolites might bind with iron and enhanced the excretion of iron, which in consequence decrease accumulation of iron and reduce the toxic effects of iron. It is quite known that hesperidin, a cranberry flavonoid act as antioxidant molecule (Chanet, A. et al., 2012), can scavenge the excess iron in biological system. High dose of Fe led to alterations in lipid metabolism and changes in the levels of serum and tissue lipids. It may be due to accumulation of Fe in liver, which plays a central role in lipid homeostasis. Iron excess induces cellular injury and functional abnormalities in hepatocytes by the process of lipid peroxidation (Britton, R. S. et al., 1987) Because the liver has a central role in the maintenance of lipid homeostasis, excess iron may alter the concentration of serum lipids, which could reduce or increase the risk of atherosclerosis. Lipid peroxidation may also damage membranes in other cells, altering important elements of control for blood pressure and heart rate. Given that increased LDL cholesterol (LDL-C) and decreased HDL cholesterol (HDL-C). Both (Brunet et al. 1999 and Dabbagh et al. 1994) found an increase in total cholesterol and triglycerides in iron over loading. The Fe^{+3} induced rise of cholesterol in serum and tissues may be due to changes in the gene expression of hepatic enzymes mainly HMG-COA reductase. Heavy metal induced change in the gene expression of HMG-COA reductase has already been reported (Kojima, Ret al., (2004). The increased plasma lipids (PLs) content in Fe intoxicated rats may be due to elevation in the levels of FFAs and cholesterol. The

antioxidant property could also contribute to the protection of membrane lipids from free radical there by cranberry's polyphenols attenuated the abnormal dispersion of membrane lipids in circulation as well as reduced the excessive generation of more toxic peroxides, which cause drastic changes in cells and tissues. Reduced risk of cardiovascular disease is often attributed to the intake phytochemicals, which lower excessive cholesterol and/or TGs concentrations (Howard, BV. and Kritchevsky, D. 1997). Lipid peroxidation is the process of oxidative degradation of poly unsaturated fatty acid and the products of lipid peroxidation inactivate cell constituents by oxidation or cause oxidative stress by undergoing radical chain reaction ultimately leading to the cell damage (Tribble, DL. and Jones, DP. 1987). Iron is the most common cofactor within the oxygen handling biological machinery and, specifically, lipid peroxidation of biological membranes is the main pathogenic mechanism of iron overload induced tissue damage (Hershko, C. et al., 1998). The mitochondrion is a target for iron toxicity, with oxidative mitochondrial damage and poisoning of enzymes of the tri carboxylic acid cycle for aerobic oxidation of glucose (Crips cycle) and energy metabolism recognized as potential targets (Harris, WR. 2002). Iron is also an essential element whose redox properties and coordination chemistry suits it for a number of catalytic and transport functions in living cells (Halliwell, B. and Gutteridge, JMC.1992). However, these same properties render iron toxic, to a large extent due to its ability to generate reactive oxygen species (Valko, et al., 2005). Iron is a well-known inducer of reactive oxygen species. Its ability to accelerate lipid peroxidation is well established (Sayre, LM. et al., 2005). Harmful effects of extreme iron deposition in liver are likely during iron overload, which has been associated with the initiation and propagation of ROS induced oxidative damage to all biomacromolecules (proteins, lipids, sugar and DNA) that can lead to a critical failure of biological functions and ultimately cell death (Butterfield, DA. and Kanski, J. 2001).

Free radicals such as superoxide anion, hydrogen peroxide, hydroxyl radical, which cause lipid peroxidation, can lead to cell death (Chen, L. and Zhang, BH. 1998). It is well known that excess free iron induces the expression of nitric oxide, releases the nitric oxide which combines with superoxide anions to form "peroxynitrite", a very toxic mediator of lipid peroxidation as well as oxidative damage to cellular membrane (Chen, L. and Wang, Y. 2001). Earlier studies have demonstrated the critical role of iron in the formation of reactive oxygen species that ultimately cause peroxidative damage to vital cell structures (Toyokuni, S. 2002). An effective therapeutic approach can play a double role in reducing the rate of oxidation one by sequestering and chelating cellular iron stores and other as radical trap (i.e., antioxidant activity) (Wilmsen, PK. et al., 2005). Since cranberry has shown antioxidant and free radical scavenging activity (Kannampalli, P. et al., 2008), the present study primarily ameliorating the effect of cranberry' polyphenols on iron accumulation and oxidative damage in the liver of iron overloaded rat is studied. Oral administration of cranberry extract significantly inverse the iron induced peroxidative damage in liver which is evidenced from the lowered levels of thiobarbituric acid reactive substances and lipid hydroperoxides. This may be due to the antioxidative effect of polyphenols (Sies, H. 1997).

5. CONCLUSION AND RECOMMENDATION

From the present study we can concluded that, the antioxidant effects of cranberry extract might be associated with the structure-antioxidant relationship of its active constituents such as vitamin C, vitamin E and polyphenols (anthocyanins).

Chelating effect of cranberry extract against liver toxicity induced by iron sulfate has not been reported earlier to our knowledge, and this study is perhaps the first observation of its kind. So, the present study showed that cranberry has a powerful hepatoprotective activity against iron sulfate induced liver toxicity. These effects could be due to membrane protective action of cranberry by scavenging the free radicals and its antioxidant action. This could serve as a stepping stone towards the discovery of newer safe and effective free radical scavenging agents.

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